bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Reagents and infection with SARS-CoV-2

Gustavo Gastão Davanzo Marcelo Hill

Updated date: Mar 27, 2023

An abbreviated version of this protocol was published in Science Advances in Sep 2022

PD-1/PD-L1 blockade abrogates a dysfunctional innate-adaptive immune axis in critical β-coronavirus disease

DOI: 10.1126/sciadv.abn6545

Detailed protocol

PBMCs Isolation

The PBMCs were obtained by using a density-gradient centrifugation technique with a 1.077 g 3 mL-1 Ficoll gradient. In this method, the Buffy coats were mixed gently and then diluted (1:1) with PBS before being carefully transferred to a 50-mL tube containing 10 mL of Ficoll. This mixture was centrifuged at 2700 rpm for 20 minutes at room temperature. The resulting PBMCs were cultured as adherent monolayers (at a concentration of 1,53106 cell3mL-1) in RPMI 1640 medium. If necessary, CD14+ cell sorting could be performed to enhance the purity of the cells. After allowing the cells to adhere for 2-3 hours, they were washed with PBS. Then they incubated in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin at 37 C with 5% CO2 atmosphere until they were ready for infection.

Viruses and Cell Lines

The SARS-CoV-2 virus stocks were propagated using the Vero cell line, and the supernatant was collected at 2-3 dpi. Plaque assays determined viral titers on Vero cells. Vero CCL-81 cells were grown in MEM medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% Penicillin-Streptomycin and were incubated at 37 C with a 5% CO2 atmosphere.

Reagents and Infection

The cells were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.1 and incubated under continuous agitation at 15 rpm for 1 hour to allow for virus absorption. Following the infection, the cells were washed twice with pre-warmed PBS and then incubated for 24 hours in RPMI medium containing 10% FBS and 1% Penicillin-Streptomycin at 37 C with a 5% CO2 atmosphere.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Gastão Davanzo, G. and Hill, M. (2023). Reagents and infection with SARS-CoV-2. Bio-protocol Preprint. bio-protocol.org/prep2185.
2. Duhalde Vega, M., Olivera, D., Gastão Davanzo, G., Bertullo, M., Noya, V., Fabiano de Souza, G., Primon Muraro, S., Castro, I., Arévalo, A. P., Crispo, M., Galliussi, G., Russo, S., Charbonnier, D., Rammauro, F., Jeldres, M., Alamón, C., Varela, V., Batthyany, C., Bollati-Fogolín, M., Oppezzo, P., Pritsch, O., Proença-Módena, J. L., Nakaya, H. I., Trias, E., Barbeito, L., Anegon, I., Cuturi, M. C., Moraes-Vieira, P., Segovia, M. and Hill, M.(2022). PD-1/PD-L1 blockade abrogates a dysfunctional innate-adaptive immune axis in critical β-coronavirus disease. Science Advances 8(38). DOI: 10.1126/sciadv.abn6545